DR MICHAEL NAGLER (Orcid ID : 0000-0003-4319-2367)

PROF. ANNE ANGELILLO-SCHERRER (Orcid ID : 0000-0003-2872-4863)

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HIGH GAS6 IN PLASMA PREDICTS VENOUS THROMBOEMBOLISM RECURRENCE, MAJOR BLEEDING AND MORTALITY IN THE ELDERLY

A PROSPECTIVE MULTICENTER COHORT STUDY

A. Schnegg-Kaufmann,[*†] S. Calzavarini,[*†] A. Limacher,[‡] M. Mean,[§¶] M. Righini,[**] D. Staub,[††] J. H. Beer,[‡‡] B. Frauchiger,[§§] J. Osterwalder,[¶¶] N. Kucher,[***] C. M. Matter,[†††] M. Husmann,[***] M. Banyai,[‡‡‡] M. Aschwanden,[††] L. Mazzolai,[§§§] O. Hugli,[¶¶¶] M. Nagler,[*†] M. Daskalakis,[*†] N. Rodondi,[§****] D. Aujesky[§] and A. Angelillo-Scherrer[*†]

[*]Department of Hematology and Central Hematology Laboratory, Inselspital, Bern University Hospital, University of Bern, Switzerland; [†]Department for BioMedical Research, University of Bern, Switzerland; ‡CTU Bern, and Institute of Social and Preventive Medicine (ISPM), University of Bern, Switzerland; §Department of General Internal Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland; ¶Department of Medicine, Lausanne University Hospital, Switzerland; [**]Division of Angiology and Hemostasis, Geneva University Hospital, Switzerland; [††]Division of Angiology, Basel University Hospital, Switzerland; ‡‡Department of Internal Medicine, Cantonal Hospital of Baden, Switzerland; [§§]Department of Internal Medicine, Cantonal Hospital of Frauenfeld, Switzerland; ¶¶Cantonal Hospital of St Gallen, Switzerland; [***]University Clinic of Angiology, University Hospital Zurich, Switzerland; [†††]Center for Molecular Cardiology, University of Zurich, and Clinic for Cardiology, University Heart Center, Zurich University Hospital, Switzerland; ‡‡‡Division of Angiology, Cantonal Hospital of Lucerne, Switzerland; [§§§]Service of Angiology, Lausanne University Hospital, Switzerland; ¶¶¶Emergency Department, Lausanne University Hospital, Switzerland; [****]Institute of Primary Health Care (BIHAM), University of Bern, Switzerland

Editorial correspondence to: Anne Angelillo-Scherrer
Department of Hematology and Central Hematology Laboratory
Inselspital
Bern University Hospital
University of Bern
CH-3010 Bern, Switzerland
phone: +41.31.632.33.02 / fax: +41 31 632 34 06
e-mail: anne.angelillo-scherrer@insel.ch

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ESSENTIALS

- Predictive ability of pro-hemostatic Gas6 for recurrent venous thromboembolism (VTE) is unknown.
- We measured Gas6 levels in 864 patients with VTE over 3 years.
- High Gas6 (>157%) at diagnosis is associated with VTE recurrence, major bleeding and mortality.
- Gas6 plasma levels measured 12 months after the index VTE are discriminatory for VTE recurrence.

SUMMARY

BACKGROUND: Growth arrest-specific gene 6 (Gas6) is a pro-hemostatic protein with an unknown predictive ability for recurrent venous thromboembolism (VTE). In the elderly, VTE carries a higher mortality but not a higher rate of VTE recurrence than in younger patients. Consequently, anticoagulation management in the elderly is challenging.

OBJECTIVE: To prospectively investigate the performance of Gas6 to predict VTE recurrence, major bleeding and mortality in the elderly.

METHODS: Consecutive patients aged ≥65 with acute VTE were followed over 3 years. Primary outcomes were symptomatic VTE recurrence, major bleeding and mortality. Plasmatic Gas6 was measured by ELISA.

RESULTS: Gas6 was measured in 864 patients at the time of the index VTE (T1) and in 70% of them, also 12 months later (T2). Gas6 at T1 was discriminatory for VTE recurrence (C-statistics: 0.56, 95% confidence interval [CI]: 0.51 to 0.62), major bleeding (0.60, 95%CI: 0.55 to 0.65) and mortality (0.69, 95%CI: 0.65-0.73) up to 36 months. VTE recurrence up to 24 months after T2 was discriminated by Gas6 at T2 (0.62, 95%CI: 0.54-0.71). High Gas6 (>157%) and continuous Gas6 levels at T1 were associated with VTE recurrence up to 6 and 12 months, respectively.

CONCLUSIONS: In elderly patients, high Gas6 is associated with a higher risk of VTE recurrence, major bleeding and death. These findings support urther studies to assess the performance of Gas6 for adjusting anticoagulation length.

INTRODUCTION

Venous thromboembolism (VTE), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), represents a worldwide major health issue and is a leading cause of cardiovascular death.(1) VTE incidence rises with age.(2-5) In the elderly population, VTE carries a higher mortality but not a higher rate of recurrence than in younger patients.(4) Elderly patients more often present comorbidities and therefore higher risk of bleeding.(4) Consequently, the management of the anticoagulation in the elderly constitutes a challenge.

Because the risk of VTE recurrence is greatest in the first 6-12 months following the initial event and progressively decreases afterwards,(6) the benefit of extended anticoagulation may be exceeded by the risk of clinically important bleeding.(7-12)

Growth arrest-specific gene 6 (Gas6), the product of the growth arrest-specific gene 6 (*GAS6*),(13) is a secreted vitamin K-dependent protein. Gas6 plasma level is known to be elevated in a variety of clinical conditions including inflammation or sepsis,(14-18) obesity,(19) chronic renal failure and cancer.(20) Importantly, no change in Gas6 plasma

level was previously observed with increasing age.(21) Nevertheless, Gas6 level progressively decreased with increasing INR under warfarin therapy.(21)

Gas6 exerts multiple functions, including regulation of cell-growth(13) and inflammation.(22) It also has effect on platelet function and coagulation, enhancing platelet aggregation and tissue factor expression in endothelial cells as well as promoting the recruitment of platelets and leukocytes to the endothelial cell membrane.(23-28) In mice, the absence of Gas6 is protective against thrombosis without causing excessive bleeding, pointing to Gas6 as an attractive target for antithrombotic therapy.(23, 25)

In a cross-sectional study including 279 patients and 79 controls, Blostein et al.(29) measured a higher Gas6 plasma level in patients 4 months after VTE than in healthy controls. In addition, they observed that subjects with elevated Gas6 level in plasma had an increased risk of VTE compared to those with lower Gas6 levels after adjustment for age, sex, medications and comorbidities. However, elevated Gas6 plasma levels were not predictive of VTE recurrence.(29) Finally, most of the patients included in this study were younger than 65 years.

Here, in a cohort of 864 patients aged 65 years or older with VTE, we prospectively investigated the performance of Gas6 plasma levels at admission and one year after the index VTE to predict the risk of VTE recurrence, major bleeding and mortality.

METHODS

Cohort sample

The study was conducted between September 2009 and December 2013 as part of the Swiss Cohort of Elderly Patients with VTE (SWITCO65+), a prospective multicenter cohort study to assess medical outcomes and quality of life in elderly patients with acute VTE from

all five university hospitals and from four high-volume non-university hospitals in Switzerland.(30)

Consecutive patients aged 65 years or older with acute VTE were identified in the in- and outpatient services of all participating study sites, and followed over 3 years. We defined deep vein thrombosis (DVT) as an acute onset of leg pain or swelling plus incomplete compressibility of a venous segment on ultrasonography or an intraluminal filling defect on contrast venography.(31)

Because iliac veins and the inferior vena cava may be technically difficult to compress, additional diagnostic criteria for iliac/caval DVT comprised abnormal duplex flow patterns compatible with thrombosis or an intraluminal filling defect on spiral computed tomography or magnetic resonance imaging venography.(32-34)

Given that ultrasonography has a reduced sensitivity and specificity for distal DVT,(35) patients with isolated distal DVT were included only if the incompressible distal deep vein transverse diameter was at least 5 mm.(36, 37)

Symptomatic pulmonary embolism (PE) was defined as an acute onset of dyspnea, chest pain, or syncope coupled with a new high-probability ventilation/perfusion lung scan; a new contrast filling defect on spiral computed tomography or pulmonary angiography; or the new documentation of a proximal DVT either by venous ultrasound or contrast venography.(37, 38) Radiographic studies used to diagnose VTE were interpreted by on-site vascular specialists or radiologists.

Exclusion criteria were inability to provide informed consent (i.e. severe dementia), conditions incompatible with follow-up (i.e. terminal illness or place of living too far from the

study center), insufficient German- or French-speaking ability, thrombosis at a site other than a lower limb, catheter-related thrombosis.

Treatment of VTE, e.g., the type of anticoagulant used (i.e. parenteral anticoagulant followed by vitamin K antagonists, parenteral anticoagulant alone, direct oral anticoagulant), the duration of the anticoagulation and the prescription of compression stocking, was entirely left to the discretion of the managing physicians.

Eligible patients were approached for informed consent to participate in the study. The ethics committees at each study site approved the study, and written informed consent was obtained from all participants. A detailed description of the study methods has previously been published.(30)

Data collection

For all enrolled patients, trained study nurses prospectively collected baseline demographic information (age, sex), type, history and complication of VTE (distal DVT, proximal DVT, overt PE, presence of post-thrombotic syndrome, prior VTE, provoked index VTE, cancer-related VTE), concomitant use of estrogen therapy during the past 3 months, immobilization during the last 3 months, major surgery during the last 3 months, comorbid conditions (history of major bleeding, chronic liver disease, renal disease, chronic or acute heart failure, cerebrovascular disease, diabetes mellitus, body mass index > 30, acute rheumatic disease during the last 3 months, inflammatory bowel disease, severe infection or sepsis during the last 3 months), high risk of fall, laboratory findings (anemia, low platelet count), concomitant use of antiplatelet drugs, arterial hypertension, heart rate ≥ 110 beats/min, systolic blood pressure < 100 mmHg, respiratory rate ≥ 30/min, temperature < 36°C, arterial oxygen saturation < 90% and VTE-related treatment using standardized data collection forms. Follow-up included one telephone interview and two face-to-face evaluations during the first year of study participation and then semi-annual contacts, alternating between face-to-face

evaluations (clinic visits or home visits in house-bound patients) and telephone calls as well as periodic reviews of the patient's hospital chart. During each visit/contact, study nurses interviewed patients to obtain information about the date and type of clinical events (recurrent VTE, bleeding, death). If a clinical event had occurred, this information was complemented by reviewing medical charts and interviewing patients' primary care physicians and family members. Collected data were recorded on standardized forms.

Blood samples

Blood was collected after minimal venostasis into 1/9 of its volume of 0.0160 M trisodium citrate (Sarstedt®) at the time of index VTE diagnosis and 12 months later.(39) Citrated platelet poor plasma (PPP) was prepared by centrifugation for 10 minutes at 2700 g and room temperature and recentrifugation of the supernatant plasma for 10 minutes at 2700 g to remove remaining platelets.(39) The resulting citrated PPP was stored in aliquots of 2 mL at -80°C within 1 hour of blood collection.(39) Citrated PPP was used for Gas6 ELISA.

Gas6 ELISA

To measure Gas6, we used the ELISA method developed by Clauser et al(40) with some modifications.(17) Wells from 96-wells plates (Maxisorp, Nunc) were coated with 100 µL per well of polyclonal goat anti-human Gas6 antibody (AB885, R&D Systems) diluted in 0.1M NaHCO3 pH 8.2 and incubated overnight at 4°C. After two washes with PBS-Tween 0.05%, 100 µL PBS-BSA 1%- sucrose 5% were added to the wells and plates were incubated 2 hours at room temperature. After three washes samples diluted 50 and 100 times and normal plasma serial dilution with PBS-BSA 1% were added to the wells, followed by an overnight incubation at 4°C. After three washes, 100 µL of biotinylated polyclonal goat antibody (BAF885, R&D Systems) were added to each well, and plates left 2 hours at room temperature. Signal was amplified with Avidin-horseradisch-peroxidase (BD Pharmingen) and plates incubated during 20 minutes at 37°C. Finally, o-phenylenediamine dihydrochloride (Sigma-Aldrich) was added. Reactions were stopped by adding 50 µL HCl

3M. Absorbance was measured at 492 nm and results were expressed in percentage relative to normal plasma, using its serial dilution as standard curve.(17, 40) This ELISA was specific for human Gas6, with no cross-reactivity with human protein S.

D-dimer

D-dimer was measured by ELISA (Vidas D-dimer exclusion test, bioMérieux).

Outcome variables

We defined objectively confirmed, symptomatic VTE recurrence, major bleeding and overall mortality up to 3 years as primary study outcomes.

VTE recurrence was defined as a fatal or new non-fatal PE or new DVT.(41) Diagnosis of recurrent VTE during follow-up was established with the following criteria: for DVT, on the basis of abnormal results on ultrasonography; and for PE, on the basis of CT or angiography showing new intraluminal defects or on the basis of ventilation-perfusion lung scan showing a high-probability pattern with new perfusion defects. A new proximal DVT, based on abnormal results on ultrasonography, associated with new PE symptom(s) (shortness of breath, chest pain, syncope) was also considered as recurrent pulmonary embolism.

Major bleeding was defined as fatal bleeding, symptomatic bleeding at critical sites (intracranial, intraspinal, intraocular, retroperitoneal, intra-articular, pericardial, or intramuscular with compartment syndrome), or a clinically overt bleeding with a reduction of hemoglobin of at least 20 g/L, or leading to transfusion of 2 or more units of packed red blood cells.(42)

We assessed the outcomes using patient or proxy interviews, interview of the patient's primary care physician, and/or hospital chart review.(30) A committee of 3 blinded clinical experts confirmed all outcomes and classified the cause of all deaths as definitely due to PE,

possibly due to PE (e.g., sudden death without obvious cause), due to major bleeding or due to another cause.(30) Final classification was made on the basis of the full consensus of this committee.(30)

Statistical analyses

We compared baseline characteristics of patients by elevated plasma Gas6 (above versus below median) using the Chi-squared test and the non-parametric Wilcoxon rank-sum test as appropriate. We calculated incidence rates of a first VTE recurrence, a first major bleeding or death up to three years after the index event by level of Gas6. Gas6 was categorized into low, medium, and high levels based on their lower and upper quartiles. We estimated the cumulative incidence of these outcomes using the Kaplan-Meier method and compared survivor functions across groups by the logrank test.

The discriminative power of Gas6 for VTE recurrence, major bleeding and mortality was assessed by Harrell's C concordance statistic.

Associations between Gas6 and the time to a first VTE recurrence and major bleeding were assessed by competing risk regression accounting for non-PE and non-bleeding related death, respectively, as a competing event, according to the method of Fine and Gray.(43) The method yields subhazard ratios (SHR) with corresponding 95%-confidence intervals (CI) and *P*-values for the failure event of primary interest. For mortality, an ordinary Cox-regression with robust standard errors was calculated. We adjusted the model for previously published predictors of VTE recurrence or major bleeding.(6, 41, 42, 44-52) For overall mortality, analyses were adjusted for age, gender, cancer, provoked VTE, prior VTE, overt PE, renal disease, history of major bleeding, heart failure, chronic lung disease, elevated heart rate, low blood pressure, low oxygen, and periods of anticoagulation as a time-varying covariate.(49, 53)

All analyses were done using Stata 14 (Stata Corporation).

RESULTS

Study sample

Of 1003 enrolled patients aged ≥ 65 years with acute VTE, we excluded 139 patients at the time of index VTE diagnosis (8 patients did not allow use of data, 4 withdrew their consent within 1 day and 127 patients had no Gas6 measurement) leaving a study sample of 864 patients (Fig. 1). Of these patients, 601 (69.6%) had Gas6 measurement 12 months after the index VTE.

Characteristics at time of index VTE diagnosis are listed in Table 1. Overall, 476 patients (44.9%) were women and the median age was 75.0 (interquartile range, IQR 69.0 ; 81.0). 599 patients (69.3 %) presented with an index PE. 251 patients (29.1%) had experienced prior VTE. 522 patients (60.4%) had unprovoked index VTE, 185 (21.4%) had provoked VTE and 157 (18.2%) had cancer-related VTE. Patients with unprovoked index VTE or with prior VTE were more susceptible to present with PE (70%) than with proximal (24%) or distal DVT only (6%). PE was more frequent in patients with unprovoked VTE (72%), than in patients with provoked (66%) or cancer related (64%) VTE (P = 0.01). Twelve months after index VTE, 432 patients (50%) were still under anticoagulation, most of them receiving vitamin K antagonists.

Gas6 plasma levels in study sample

At the time of index VTE diagnosis, median Gas6 level was 129.3% (IQR 108.9, 156.6) (T1 on Fig. 2). Patients with elevated Gas6 level (> 129%) at the time of index VTE were slightly older (median age 76 *versus* 74, P = 0.001). However, the correlation between Gas6 level and age was weak both at the time of the index VTE (Spearman correlation, r_s = 0.12) and 12 months later (r_s = 0.09). Patients with elevated Gas6 at the time of the index VTE diagnosis were more likely to have cancer-related VTE. They were also more immobilized

during the last 3 months and displayed a higher prevalence of post-thrombotic syndrome, history of major bleeding, anemia, thrombocytopenia, heart rate ≥ 110 beats/min and oxygen saturation < 90% (Table 1). In contrast, these patients were less likely to be still under oral anticoagulation 12 months after index VTE (180 [41.4%] patients with Gas6 above median *versus* 230 [53.6%] patients with Gas6 below or at median, $P < 0.001$). Interestingly, patients with inflammatory bowel disease were more likely to have a lower Gas6 level ($P = 0.016$). Twelve months after the index VTE, median Gas6 level was 93% (IQR 77.1, 111.7) (T2 on Fig. 2). However, at this time point, median Gas6 was lower in patients on than in patients off anticoagulation (86.1% [IQR 70.4, 107.3] versus 100.2% [IQR 85.3, 116.8], $P < 0.001$).

Gas6 plasma levels were generally lower 12 months after the index VTE (T2) than those at the time of the index VTE (T1' versus T2, $P < 0.001$, Fig. 2). In a minority of patients ($n = 97$, 11%), Gas6 increased from VTE diagnosis to 12 months after.

The correlation between Gas6 and D-dimer was weak, both at time of the index VTE (Spearman correlation, $r_s = 0.06$) and 12 months later ($r_s = 0.24$).

Incidence of VTE recurrence, major bleeding and mortality

After a follow-up of 3 years, 100 patients had developed recurrent VTE, resulting in an incidence rate of 5.6 per 100 person-years (95% confidence interval [CI] 4.6 to 6.8). During the same period, 170 of 864 patients had died (mortality rate 9.0 per 100 person-years; 95% CI 7.8 to 10.5). Mortality rate was higher during the initial 6 months whereas VTE recurrence rate remained stable over the observation-period (Table S1). During the whole follow-up, incidence rates of VTE recurrence and major bleeding were higher in patients with high Gas6 level than in patients with medium or low Gas6 levels measured at the time of the index VTE (Table S1). Likewise, the 2-year cumulative incidence of VTE recurrence was higher for patients with high (> 157%) versus medium (109-157%) and low (< 109%) Gas6 levels measured at the time of the index VTE, however not significantly ($P = 0.087$) (Fig. 3A).

The 2-year cumulative incidence of major bleeding was higher for patients with high (> 157%) versus medium (109-157%) and low (< 109%) Gas6 levels measured at the time of the index VTE (P = 0.0004) (Fig. 3B).

The 2-year cumulative incidence of overall mortality was 7%, 15% and 35% (P < 0.001) for patients with a low, medium and high Gas6 level, respectively (Fig. 3C).

Discriminative power of Gas6 levels for outcomes

In order to evaluate the discriminative power of Gas6 levels, C-statistic (95%-CI) values were calculated (Table 2). Gas6 level measured at the time of the index VTE were discriminatory for VTE recurrence, major bleeding and mortality up to 36 months.

Gas6 level measured 12 months after the index VTE was discriminatory for VTE recurrence up 24 months. In contrast, when measured 12 months later, Gas6 level was not discriminatory for major bleeding and mortality up to 24 months (Table 2).

Association between Gas6 plasma level and outcomes

High Gas6 levels (> 157%) measured at the time of the index VTE were associated with increased risk of VTE recurrence up to 6 months (Table 3), and major bleeding up to 36 months (crude analysis) (Table 4) . In continuous analysis (log-transformed Gas6 levels), the risk of VTE-recurrence was increased up to 12 months (Table 3), and the risk of major bleeding up to 36 months (crude analysis) (Table 4).

In addition, medium (109-157%) and high Gas6 levels were associated with increased overall mortality up to 36 months (Table 5).

These associations also remained after adjustment for potential confounding factors for the risk of VTE recurrence and overall mortality (Table 3 and 5).

Regarding the risk of major bleeding, only the association with high Gas6 measured at the time of the index VTE remained up to 6 months after adjustment for potential confounding factors (Table 4).

We assessed the relationship between continuous log-transformed Gas6 values and hazards of VTE recurrence and overall mortality using fractional polynomial competing risk and Cox proportional hazards models, which showed that (sub)-hazards augmented with increased Gas6 values in a linear way (Supplementary Figure).

The findings of the sensitivity analyses revealed that these associations also remained after the exclusion of patients with cancer (Table S2 or with cancer and provoked VTE (Table S3). Moreover, in the subgroup of patients off of oral anticoagulation 12 months after the index VTE, continuous (log-transformed) Gas6 levels were associated with VTE recurrence up to 12 months (Table S4). This association also remained after adjustment for potential confounding factors (Table S5). Finally, medium, high and continuous (log-transformed) Gas6 levels were associated with increased mortality up to 36 months (Table 5).

DISCUSSION

We followed prospectively 864 elderly patients with VTE over 3 years and observed that patients with higher Gas6 were more likely to have cancer-related VTE and co-morbidities. Our findings are consistent with previous publications reporting high Gas6 levels in a number of clinical conditions, most of them associated with inflammation and organ damage (14, 17, 54, 55).

Our data showed that elevated Gas6 was independently associated with recurrent VTE up to 12 months, with major bleeding up to 6 months and with mortality up to 36 months after the index VTE. Considering that patients with more comorbidities were more likely to have

higher Gas6 levels, neither the association with VTE recurrence, major bleeding nor overall mortality was surprising. However, the observed association remained significant after adjustment for a large number of comorbidities (Table 3-5). Gas6 was also still associated with VTE recurrence and mortality after the exclusion of patients with cancer (Table S2) or with cancer and provoked VTE (Table S3). Because Gas6 is a pro-hemostatic protein (23-25), we may presume that the association between high Gas6 levels and VTE recurrence might be at least partly causal.

Another important finding of this study is that Gas6 levels measured at the time of diagnosis were discriminatory for VTE recurrence and mortality. In addition, Gas6 levels measured 12 months after the index VTE were discriminatory only for VTE recurrence. A previous study comprising a lower number of patients than this study did not demonstrate the predictive ability of Gas6 for VTE recurrence (29).Thus, the data of the present study point to elevated Gas6 level as an independent predictor for VTE recurrence, major bleeding and mortality up to 36 months in the elderly. Gas6 might be then useful in adjusting the intensity of surveillance in this group of high-risk patients. However, before considering Gas6 as an additional marker to predict recurrence and guide therapy, Gas6 level would need to be compared with or integrated in established risk scores such as DASH,(56) HERDOO-2,(57) Vienna(58) scores.

Our study has some limitations. First, the spectrum of the study was limited to elderly patients and 18.2% of them had cancer, the mortality from comorbid diseases is naturally higher than the VTE recurrence rate, as persons with limited life expectancy often do not have the time to develop recurrent VTE. Thus, it is indeed unclear whether the results can be extrapolated to younger persons with VTE. In addition, although Gas6 plasma level was previously reported not to be influenced by age,(21) its prediction ability for VTE recurrence as well as its association with VTE recurrence would need to be studied in younger patients. Second, Gas6 was previously reported to be elevated in several other medical conditions.

Nevertheless, in this study, we were able to demonstrate that the association between Gas6 and VTE recurrence and mortality remained after adjustment for these conditions. However, this needs to be verified in younger patients. Third, VTE treatment has changed since this cohort has been constituted, i.e. direct oral anticoagulants have replaced vitamin K antagonists in most patients. Therefore, it is unclear whether the results can be extrapolated to patients treated by direct oral anticoagulants. Fourth, as we have enrolled patients with VTE in in- and outpatients hospital services, the proportion of patients with pulmonary embolism was relatively high and represented 69% of our study sample. Fifth, Gas6 testing was performed only at the time of the index VTE and 12 months later, when about 50% of the patients were still under oral anticoagulation. Because we and others(21) demonstrated that Gas6 levels are affected by oral anticoagulation by vitamin K antagonists, we can assume that the significantly lower Gas6 level 12 months after the index VTE was at least partly due to the antivitamin K effect. Interestingly, Gas6 levels in the subgroup of patients off of oral anticoagulation at this time point were significantly lower than those of patients on anticoagulation. Thus, the correct interpretation of Gas6 levels would require that patients interrupt anticoagulation, exposing those with increased risk to the possibility of a VTE recurrence. Finally, even though we adjusted our analyses for many covariates, we might have missed important predictor variables.

In conclusion, in the elderly, high Gas6 is associated with a higher risk of VTE recurrence, major bleeding but only up to 6 months, a period of time where most patients were still anticoagulated, and death. Our data suggest that a clinical decision to avoid prolonged anticoagulation could be attempted based on Gas6 plasma level in the elderly. Further studies are required to confirm whether the use of Gas6 levels for adjusting anticoagulation length leads to better outcomes, especially in younger patients.

ADDENDUM

A. Schnegg-Kaufmann, S. Calzavarini and A. Angelillo-Scherrer designed the protocol and the analyses plan, conducted the analyses and drafted the manuscript. S. Calzavarini performed Gas6 measurements. A. Limacher performed the statistical analysis. A. Schnegg-Kaufmann, S. Calzavarini and A. Angelillo-Scherrer interpreted the data. M. Méan, M. Righini, B. Frauchiger, J. Osterwalder, N. Kucher and N. Rodondi organized data collection, intellectually reviewed the manuscript, and participated in funding procedures. A. Schnegg-Kaufmann, S. Calzavarini, A. Limacher, D. Staub, J.H. Beer, C.M. Matter, M. Husmann, M. Banyai, M. Aschwanden, L. Mazzolai, O. Hugli, M. Nagler and M. Daskalakis organized data collection and intellectually reviewed the manuscript. D. Aujesky was principal investigator of the SWITCO65+ cohort and was responsible for planning of the study, data collection, drafting of the manuscript and obtaining funding. A. Angelillo-Scherrer was in charge of the Gas6 nested study and was responsible for planning of the study, data collection, drafting of the manuscript and obtaining funding. All authors approved the final version of the manuscript.

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DISCLOSURE OF CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Table S1. Incidence rates of VTE recurrence, major bleeding and mortality rates by level of Gas6 measured at the time of the index VTE

Table S2. Sensitivity analyses: from baseline onward using baseline Gas6, excluding patients with cancer

Table S3. Sensitivity analyses: from baseline onward using baseline Gas6, excluding patients with cancer and provoked VTE

Table S4. Association between Gas6 measured 12 months after the index VTE in patients off oral anticoagulation and VTE recurrence from 12 months after the index VTE onward

Supplementary Figure: Relative subhazards for VTE recurrence and relative hazards for overall mortality

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FIGURE LEGENDS

Figure 1. Flow diagram of patients included in the study

VTE, venous thromboembolism

Figure 2. Gas6 plasma levels at the time of VTE diagnosis and 12 months later.

Box-plot of Gas6 levels presented as median with interquartile range (IQR) and whiskers with maximum length of 1.5 IQR. T1, Gas6 level at the time of the index VTE of all patients (Gas6: *n* = 864). T1' Gas6 level at the time of the index VTE of patients who also had Gas6 level measured at T2 (Gas6: *n* = 601). T2, Gas6 level 12 months after the index VTE (Gas6: *n* = 601). The *P* value compared T1' and T2 and is from a Wilcoxon matched pairs signed-ranks test. The Spearman correlation between T1' and T2 was $r_s = 0.33$ for Gas6.

Figure 3. Cumulative incidence of VTE, major bleedingand mortality for strata of Gas6.

The cumulative incidence of VTE (A), major bleeding (B) and mortality (C) for strata of Gas6 levels was estimated using the Kaplan-Meier method and compared survivor functions across groups by the logrank test. Gas6 levels were categorized based on the lower and upper quartile as low (< 109%), medium (109-157%) and high (> 157%).

TABLES

Table 1. Patients characteristics at the time of the index VTE by Gas6 plasma level (above *versus* below or at median)

Characteristic[*]	All	Gas6 above median (>129%)	Gas6 below or at median (≤129%)	*P* value
		n (%) or median (IQR)	n (%) or median (IQR)	
Total number of patients, n	n = 864	n = 435	n = 429	
Patient age, years	75.0 (69.0; 81.0)	76.0 (70.0; 82.0)	74.0 (69.0; 80.0)	0.001
Sex (women)	388 (44.9%)	207 (47.6%)	181 (42.2%)	0.111
VTE location				0.053
distal DVT only	70 (8.1%)	29 (6.7%)	41 (9.6%)	
proximal DVT	195 (22.6%)	111 (25.5%)	84 (19.6%)	
pulmonary embolism	599 (69.3%)	295 (67.8%)	304 (70.9%)	
Type of VTE†				<0.001
unprovoked	522 (60.4%)	242 (55.6%)	280 (65.3%)	
provoked	185 (21.4%)	92 (21.1%)	93 (21.7%)	
cancer-related	157 (18.2%)	101 (23.2%)	56 (13.1%)	
Current oestrogen therapy during the last 3 months[*]	27 (3.1%)	9 (2.1%)	18 (4.2%)	0.073
Immobilization during the last 3 months	190 (22.0%)	115 (26.4%)	75 (17.5%)	0.001
Major surgery during the last 3 months	131 (15.2%)	72 (16.6%)	59 (13.8%)	0.251
Prior VTE	251 (29.1%)	125 (28.7%)	126 (29.4%)	0.837
Presence of PTS[*,‡]	453 (52.4%)	251 (57.7%)	202 (47.1%)	0.003
History of major bleeding[*]	89 (10.3%)	54 (12.4%)	35 (8.2%)	0.039
Chronic liver disease	13 (1.5%)	10 (2.3%)	3 (0.7%)	0.053
Renal disease[§]	170 (19.7%)	97 (22.3%)	73 (17.0%)	0.051
Chronic or acute heart failure	103 (11.9%)	57 (13.1%)	46 (10.7%)	0.280
Cerebrovascular disease (stroke, TIA)	84 (9.7%)	44 (10.1%)	40 (9.3%)	0.695
Diabetes mellitus	137 (15.9%)	79 (18.2%)	58 (13.5%)	0.062
BMI >30[*]	201 (23.3%)	107 (24.6%)	94 (21.9%)	0.360
High risk of fall[*,‖]	406 (47.0%)	233 (53.6%)	173 (40.3%)	<0.001
Acute rheumatic disease during the last 3 months	29 (3.4%)	17 (3.9%)	12 (2.8%)	0.365
Inflammatory bowel disease	31 (3.6%)	9 (2.1%)	22 (5.1%)	0.016
Severe infection or sepsis during the last 3 months	71 (8.2%)	42 (9.7%)	29 (6.8%)	0.121
Anemia[*,¶]	335 (38.8%)	206 (47.4%)	129 (30.1%)	<0.001
Platelet count <150 G/l[*]	132 (15.3%)	78 (17.9%)	54 (12.6%)	0.039
Antiplatelet therapy[#]	275 (31.8%)	147 (33.8%)	128 (29.8%)	0.212
Arterial hypertension	552 (63.9%)	289 (66.4%)	263 (61.3%)	0.116
Heart rate ≥110 beats/min[*]	79 (9.1%)	49 (11.3%)	30 (7.0%)	0.031

Systolic BP <100 mmHg*	28 (3.2%)	13 (3.0%)	15 (3.5%)	0.664
Respiratory rate ≥30/min*	28 (3.2%)	16 (3.7%)	12 (2.8%)	0.469
Temperature <36°C*	65 (7.5%)	27 (6.2%)	38 (8.9%)	0.119
Arterial oxygen saturation <90%*	93 (10.8%)	62 (14.3%)	31 (7.2%)	0.001

Abbreviations: BP, blood pressure; BMI, body mass index; DVT, deep vein thrombosis; IQR, interquartile range, PTS, post-thrombotic syndrome; TIA, transient ischemic attack; VTE, venous thromboembolism.

* Values were missing forestrogen therapy during the last 3 months (0.1%), presence of PTS (1.9%), history of major bleeding (0.1%), BMI>30 (0.6%), high risk of fall (0.1%), anemia (5.8%), platelet count (5.8%), heart rate ≥110 beats/min (2.1%), systolic BP <100 mmHg (1.6%), respiratory rate ≥30/min (21.1%), temperature <36°C (7.8%), arterial oxygen saturation <90% (21.3%).

† Provoked VTE is defined as immobilization, surgery, or estrogen therapy during last three months. Cancer is defined as any solid or hematologic cancer that required chemotherapy, radiation therapy, surgical treatment, or palliative treatment during the last 3 months.

‡ Defined as Villalta score >5 or presence of an ulcer on left or right side

§ Chronic renal disease or creatinine clearance <30 mL/min

‖ Defined as answering yes to at least one screening questions: 1) Did you fall during the last year?; 2) Did you notice any problem with gait, balance, or mobility?

¶ Anemia= Hemoglobin <12g/dL for female or <13g/dL for male

Defined as antiplatelet therapy such as aspirin 100 to 300 mg daily, clopidogrel, prasugrel, or spirin/dipyridamol at the time of the index VTE

Table 2. Discriminative power of Gas6 plasma level for outcomes

From the time of the index VTE (T1) onward using measurements performed at the time of VTE diagnosis (T1)			
	No events/ no patients	**C-statistics (95% confidence interval)**	***P* value***
Gas6 at the time of VTE diagnosis			
VTE recurrence			
Up to 6 months	24/864	0.67 (0.57 to 0.78)	0.001
Up to 12 months	48/864	0.61 (0.52 to 0.69)	0.010
Up to 24 months	83/864	0.58 (0.52 to 0.64)	0.010
Up to 36 months	100/864	0.56 (0.51 to 0.62)	0.031
Major bleeding			
Up to 6 months	62/864	0.62 (0.55 to 0.69)	<0.001
Up to 12 months	82/864	0.60 (0.54 to 0.66)	0.001
Up to 24 months	103/864	0.60 (0.55 to 0.65)	<0.001
Up to 36 months	118/864	0.60 (0.55 to 0.65)	<0.001
Overall mortality			
Up to 6 months	77/864	0.73 (0.67 to 0.78)	<0.001
Up to 12 months	97/864	0.71 (0.65 to 0.76)	<0.001
Up to 24 months	149/864	0.70 (0.66 to 0.74)	<0.001
Up to 36 months	170/864	0.69 (0.65 to 0.73)	<0.001

From 12 months after the index VTE (T2) onward using measurements performed 12 months after the index VTE (T2)			
	No events/ no patients	**C-statistics (95% confidence interval)**	***P* value***
Gas6 12 months after the index VTE			
VTE recurrence			
Up to 12 months	32/601	0.66 (0.56 to 0.75)	0.002
Up to 24 months	49/601	0.62 (0.54 to 0.71)	0.003
Major bleeding			
Up to 12 months	18/601	0.58 (0.43 to 0.72)	0.294
Up to 24 months	32/601	0.57 (0.47 to 0.68)	0.173
Overall mortality			
Up to 12 months	33/601	0.57 (0.47 to 0.68)	0.181
Up to 24 months	48/601	0.56 (0.48 to 0.65)	0.159

Abbreviations: VTE, venous thromboembolism
*The p-value is from a test of the null hypothesis of no discrimination (i.e. a c-statistics of 0.5).

Table 3. Association between Gas6 plasma level and VTE recurrence - from the time of the index VTE (T1) onward using Gas6 measured at the time of VTE diagnosis (T1)

		n/N (%)	Crude subhazard ratio (95% confidence interval)	*P* value	Adjusted subhazard ratio (95% confidence interval)	*P* value
Up to 6 months						
Gas6 at the time of the index VTE (categorized)	Low (<109%)	2/216 (0.9)	Reference		Reference	
	Medium (109-157%)	11/435 (2.5)	2.77 (0.61 to 12.51)	0.185	2.95 (0.62 to 13.95)	0.172
	High (>157%)	11/213 (5.2)	5.74 (1.27 to 25.95)	0.023	6.65 (1.44 to 30.80)	0.015
Log-transformed Gas6 at the time of the index VTE	Continuous (per log-unit)	24/864 (2.8)	4.71 (1.98 to 11.19)	<0.001	5.04 (2.14 to 11.88)	<0.001
Up to 12 months						
Gas6 at the time of the index VTE (categorized)	Low (<109%)	8/216 (3.7)	Reference		Reference	
	Medium (109-157%)	23/435 (5.3)	1.46 (0.65 to 3.25)	0.355	1.50 (0.66 to 3.40)	0.335
	High (>157%)	17/213 (8.0)	2.26 (0.98 to 5.23)	0.056	2.42 (1.00 to 5.89)	0.051
Log-transformed Gas6 at the time of the index VTE	Continuous (per log-unit)	48/864 (5.6)	2.42 (1.12 to 5.24)	0.025	2.47 (1.08 to 5.64)	0.032

Abbreviations: PE, pulmonary embolism; VTE, venous thromboembolism
Adjustments: VTE recurrence was adjusted for age, cancer, provoked VTE, prior VTE, overt PE, renal disease,
and periods of AC (oral or parenteral anticoagulation) as a time-varying covariate.(6, 41, 44-52)

Table 4. Association between Gas6 plasma level and major bleeding up to 6 months

		n/N (%)	Crude SHR (95%-CI)	*P* value	Adjusted SHR (95%-CI)	*P* value
From the time of the index VTE (T1) onward using Gas6 measured at the time of VTE diagnosis (T1)						
Gas6 at the time of the index VTE (categorized)	Low (<109%)	7/216 (3.2)	Reference		Reference	
	Medium (109-157%)	32/435 (7.4)	2.33 (1.03 to 5.28)	0.043	2.07 (0.89 to 4.82)	0.093
	High (>157%)	23/213 (10.8)	3.47 (1.49 to 8.10)	0.004	2.58 (1.04 to 6.37)	0.040
Log-transformed Gas6 at the time of the index VTE	Continuous (per log-unit)	62/864 (7.2)	2.79 (1.42 to 5.46)	0.003	2.05 (0.95 to 4.41)	0.067

Abbreviation: VTE, venous thromboembolism
Adjustments: Major bleeding was adjusted for age, cancer, provoked VTE, prior VTE, overt PE, renal disease, history of major bleeding, anemia, antiplatelet therapy, and periods of AC as a time-varying covariate.(51, 59-73)

Table 5. Association between Gas6 plasma level and overall mortality up to 36 months

		n/N (%)	Crude hazard ratio (95% confidence interval)	*P* value	Adjusted hazard ratio (95% confidence interval)	*P* value
From the time of the index VTE (T1) onward using Gas6 measured at the time of VTE diagnosis (T1)						
Gas6 at the time of the index VTE (categorized)	Low (<109%)	20/216 (9.3)	Reference		Reference	
	Medium (109-157%)	73/435 (16.8)	1.96 (1.20 to 3.19)	0.007	1.69 (1.00 to 2.84)	0.048
	High (>157%)	77/213 (36.2)	4.95 (3.04 to 8.05)	<0.001	3.44 (2.03 to 5.82)	<0.001
Log-transformed Gas6 at the time of the index VTE	Continuous (per log-unit)	170/864 (19.7)	7.21 (4.48 to 11.60)	<0.001	5.00 (3.16 to 7.92)	<0.001
From the time of the index VTE onward using Gas6 as a time-varying covariate (at the time of the index VTE and 12 months later)						
Gas6 time-varying covariate (categorized)	Low (<109%)		Reference		Reference	
	Medium (109-157%)		1.88 (1.26 to 2.80)	0.002	1.68 (1.09 to 2.57)	0.017
	High (>157%)		5.55 (3.63 to 8.47)	<0.001	3.55 (2.21 to 5.71)	<0.001
Log-transformed Gas6 time-varying covariate	Continuous (per log-unit)		8.50 (5.51 to 13.11)	<0.001	5.18 (3.17 to 8.46)	<0.001

Abbreviations: VTE, venous thromboembolism
Adjustments: Mortality was adjusted for age, gender, cancer, provoked VTE, prior VTE, overt PE, renal disease, history of major bleeding, heart failure, chronic lung disease, high pulse, low blood pressure, low oxygen, and periods of anticoagulation as a time-varying covariate.(49, 53)

Figure 1

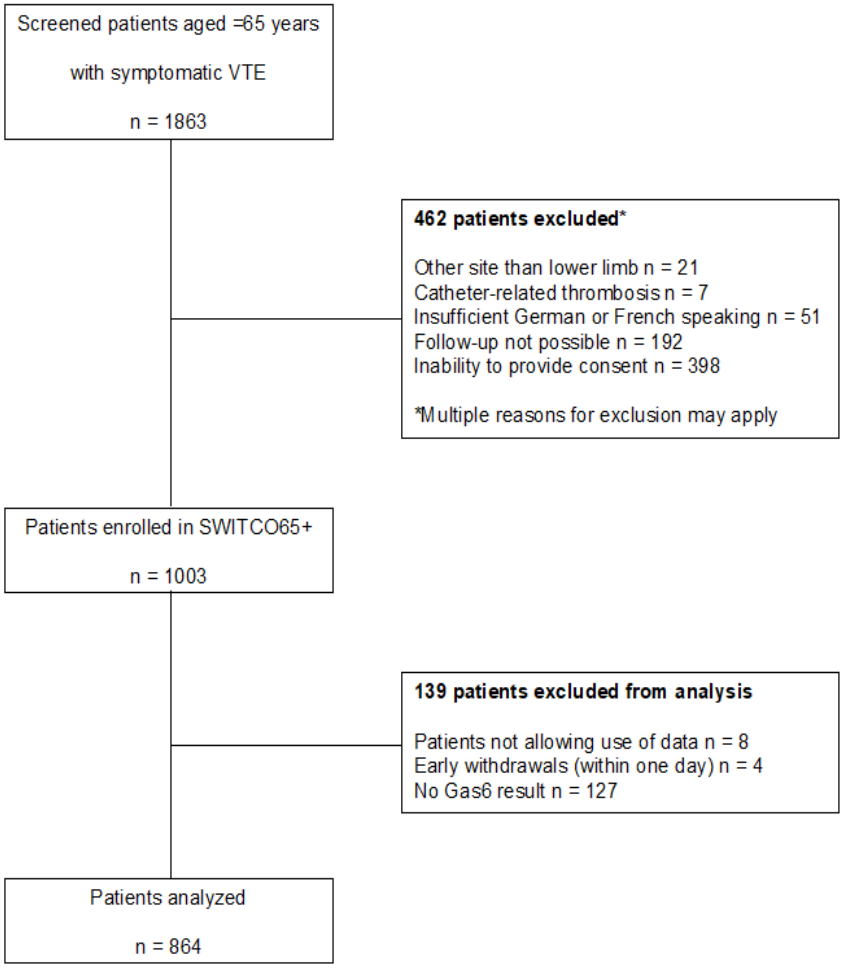
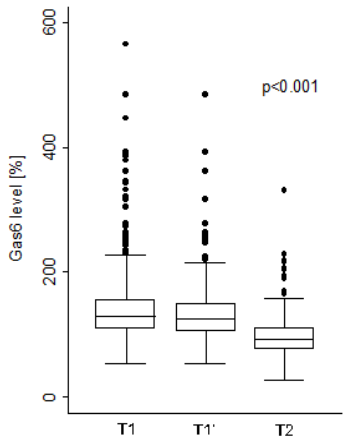


Figure 2

A



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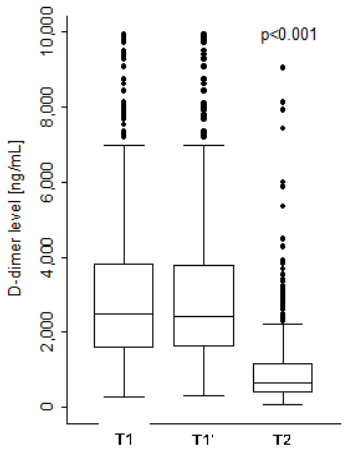
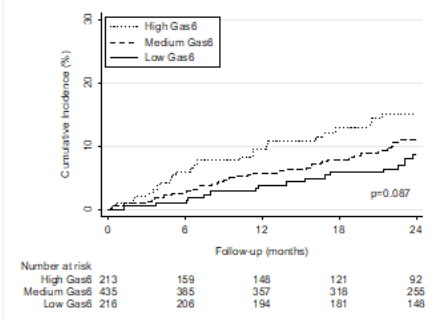


Figure 3

A



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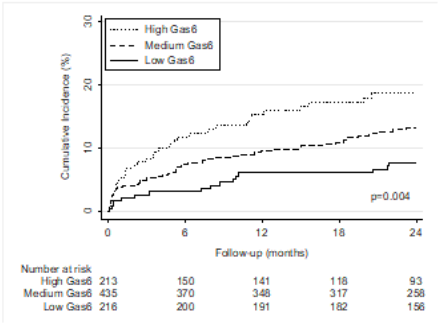

C